Effect of Irrigation on Growth and Development of the Root System of Two Medicinal Plants, *Hyssopus officinalis* and *Passiflora incarnata*

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Abstract

Medicinal and aromatic plants are known to be used by a large portion of global population for their medicinal therapeutic effects. Water is among the most important factors for the development, yield and quality of medicinal plants since its deficiency may cause serious growth damages and yield losses. The objective of the present study was to evaluate the effect of two irrigation rates on the development and characteristics of the root system of medicinal plants *Hyssopus officinalis* L. and *Passiflora incarnata* L. The experiments were conducted in 2013 in western Greece in the region of Agrinio according to completely randomized design. The root parameters that were determined were root density, root diameter, root surface and root volume, while some soil parameters and abscular mycorrhiza colonization were also measured. The results indicated that for both medicinal plants the increase of irrigation rate had a positive effect on density and volume of the root system, and the other characteristics of the root correlated with the growth and the forage yield of the plants. In conclusion, irrigation can positively affect the growth and development of the root system of *H. officinalis* and *P. incarnate* with a beneficial impact on overall plant growth and productivity.

Keywords: *Hyssopus officinalis, Passiflora incarnata, irrigation, medicinal plants, root characteristics.*

INTRODUCTION

Hyssop (*Hyssopus officinalis* L.) is a perennial herb cultivated in Asia, America (Pandey *et al*, 2014) and Europe (Kindlovits *et al.*, 2014). Hyssop can be cultivated as essential oil crop. Kara and Baydar (2012) reported that the main components of hyssop essential oils were sabinene (28.4-57.2%), heneicosene (9.49-37.0%), geranyl acetate (7.5-25.0%). In addition, Zheljazkov *et al.* (2012) reported that the main constituent of hyssop essential oils was pinocamphene+isopinocamphene (57-75%). Also, hyssop extracts shows antifungal and antioxidant activity (Džamić *et al.*, 2013).

Moreover, large potential exists to use passion flower (*Passiflora incarnata* L.) as medicinal plant. Passion flower is known to show sedative, antitussive, antiasthmatic, and antidiabetic activities (Krenn, 2002; Miroddi *et al.*, 2013). Passion flower is also used for the treatment of some diseases like as mild depression, anxiety (Fiebich *et al.*, 2011), insomnia, convulsion, sexual dysfunction and cancer (Patel *et al.*, 2009). The main compounds in passion flower plants are vitexin (Draganova, 2014), isovitexin and swertisin (Wohlmuth *et al.*, 2010).

Furthermore, limited data are available for crop production technique for hyssop and passion flower. McGuire (1998) observed that *P. incarnata* plants grown in the field in New York State flowered throughout the summer and were pollinated by carpenter bees, but fruit set was low without hand-pollination. Kara and Baydar (2012) reported that the highest essential oil yield (9.2 kg/ha) was obtained from hyssop plants at full blooming stage. Furthermore, Kizil *et al.* (2008) observed that the concentration of essential oils in hyssop plants is affected by environmental conditions. According to Moro *et al.* (2011) the hyssop crop should be irrigated. Limited data are available regarding the irrigation water requirements of *H. officinalis* and *P. incarnata* crops. Therefore, the objective of our study was to evaluate the effect of two irrigation levels on the development and characteristics of the root system of the medicinal plants *Hyssopus officinalis* and *Passiflora incarnata*.

MATERIALS AND METHODS

The experiment fields were settled in western Greece in the region of city Agrinio (38°38'08.43'', 21°21'16.62'') at June of 2013. It was established in the 'organic' experimental field of ASKAFEFA cooperative union and certificated by TUV Austria (EN 834/2007). The soil was a clay loam (24.9% clay, 61.2% silt, and 13.9% sand) with pH 7.6 and EC 0.63 mS cm^{-1}. The seedlings placed at densities 75 X 35cm for *Hyssopus officinalis* and 75 X 50cm for *Passiflora incarnata*. The experimental design was completely randomized with four replications. Each irrigation had two levels (normal and double dose). The normal irrigation dose (IR1) was 15 mm and the double irrigation dose (IR2) was 30 mm of water. The irrigations were conducted by means of a drip irrigation system. In total, there were 10 and 8 irrigations per year for *Hyssopus officinalis* and *Passiflora incarnate*, respectively. The plot size was 3 m x 8 m. Weeds were controlled by hand, with two hoeings being carried out.

The parameters that were determined were: root length density (RLD), root mass density (RMD), root surface density (RSD), abscular mycorrhiza (AMF), soil porosity (SP) , penetration resistance (PR), soil mean weight diameter (MWD). Root samples were collected at the of July 2014, at flowering stage, and from the 0–35 cm layer by using a cylindrical auger (25 cm length, 10 cm diameter) at the midpoint between successive plants within a row. Firstly, roots were separated from the soil by soaking the samples overnight in 30 ml of a 0.5% solution of sodium hexametaphosphate. Afterwards, the samples were stirred for 5 min and washed over a 5 mm mesh-sieve. The roots thus held on the sieves were decanted into a 0.1% trypan blue FAA staining solution (mixture of 10% formalin, 50% ethanol and 5% acetic acid solutions). For the determination of root length density (RLD) and root surface (RS), the stained root samples were placed on a high resolution scanner (Hewlett Packard 4c, Palo Alto, CA, USA) and images captured using Delta-T software was used, (Delta-T Scan version 2.04; Delta-T Devices Ltd, Burwell, Cambridge, UK). The root dry mass density (RMD) was determined after drying for 48 h at 70 °C.

The second root samples were cleaned and stained with trypan blue in lactophenol, according to the method of Phillips and Hayman (1970). The percentage of root length colonized by AM fungi was determined microscopically with the gridline-intersection method at a magnification of × 30-40 (Giovannetti and Mosse, 1980). Total porosity of the soil was determined by 1-Db/Dp, where Dp is the particle density (2.5 g cm^{-3}) and D_b is the soil bulk density. Soil bulk density was determined for each plot by taking undisturbed soil cores with 100 cm^3-cylinders from a depth of 0-10 cm.

Mean weight diameter (MWD) of aggregates was determined by using the oscillation apparatus Analysette 3, (Spartan, Fritsch Ltd, Oberstein, Germany) at flowering stage. The oscillation time was 3 min using 1 liter of fresh soil from 0 to 10 cm depth and mesh sizes of 20 to 40, 10 to 20, 5 to 10, 2 to 5, and <2 mm. After separation of each aggregate class, the soil was dried at 105 °C. The MWD equals the sum of the products of the mean diameter, x_i, of each size fraction and the proportional weight, w_i, of the corresponding size fraction. For calculating the MWD, the following equation was used (Van Bavel, 1949).

$$MWD = \sum_{i=1}^{n} x_i w_i \quad (1)$$

Penetration resistance of the soil was measured using a digital penetrologger (model 06.15; Eijkelkamp Equipment Ltd, Giesbeek,

Tab. 1. Effect of irrigation dose on soil and root properties of *Hyssopus officinalis.* F values and p-levels are also shown.

	Soil Porosity %	Penetration Resistance MPa	MWD (mm)	RMD g/ cm^3	RLD cm/ cm^3	Root surface mm^2/cm^3	AMF %
IR1	40.00	2.175	9.225	0.113	0.270	0.855	22.00
IR2	42.75	1.852	10.75	0.137	0.365	1.227	27.75
F value	8.44	11.80	15.19	13.45	5.13	12.16	9.28
p level	0.027	0.014	0.008	0.010	ns	0.013	0.023

Tab. 2. Effect of irrigation dose on soil and root properties of *Passiflora incarnate*. F values and p-levels are also shown.

	Soil Porosity %	Penetration Resistance MPa	MWD (mm)	RMD g/ cm^3	RLD cm/ cm^3	Root surface mm^2/cm^3	AMF %
IR1	39.75	2.16	10.05	0.097	0.224	0.796	39.50
IR2	43.00	1.81	12.05	0.121	0.284	1.013	47.00
F-value	6.76	11.13	9.88	27.93	3.38	23.09	29.35
p level	0.041	0.016	0.020	0.002	ns	0.003	0.002

The Netherlands). Penetration resistance was determined in all plots at depth intervals of 1 cm within the 0–30 cm layer. Then, average values were calculated.

For analysis of variance (ANOVA), comparisons of means and 3D graphs, the software Statsoft (2007) was used. All comparisons were made at the 5% level of significance.

RESULTS AND DISCUSSION

The results of the present study indicated that for both medicinal plants (*Hyssopus officinalis* and *Passiflora incarnata*) the use of irrigation had a positive effect on both, density and volume of the root system (Tab. 1 and 2). Our results indicated that the highest hyssop root mass density and root length density was observed for IR2 treatment (0.137 g/cm^3 and 0.365 cm/cm^3, respectively). Moreover, limited data are available regarding the irrigation water requirements of *H. officinalis* and *P. incarnata* crops. Khazaie *et al.* (2008) reported that the irrigation intervals (7, 14 and 21 days) and plant density (5, 6.6 and 8 plants m^{-2}) did not change biomass and oil production of hyssop crop. Generally, medicinal plants are resistant to drought conditions but under Mediterranean conditions these plants should be irrigated to maximize yields. According to Ekren *et al.* (2012) the irrigation of purple basil (*Ocimum basilicum* L.) resulted in an increase of crop yield. Atallah *et al.* (2011) also reported that herbage and essential oils yield of oregano (*Origanum syriacum* L.) were influenced by irrigation regime.

Moreover, *P. incarnata* crop, the highest root mass density and root length density were again found in IR2 treatment (0.284 g/cm^3 and 1.013 cm/cm^3, respectively). The root length density and root surface density was lower in *P. incaranta* than that in *H. officinalis* crop.

Correlations between most of the soil and root parameters were statistically significant for both plant species, as shown in Tab. 3 and 4. The root surface density of hyssop plants had positive and significant correlation (Tab. 3) with soil porosity ($r=0.75$, $p<0.05$). In addition, root mass density had negative and significant correlation with penetration resistance of soil ($r=-0.81$, $p<0.01$ and $r=0.93$, $p<0.001$, for hyssop and passion flower crop, respectively). Furthermore, in order to evaluate the joint effects of some of the studied parameters, multiple regression analysis was conducted. In the case of *H. officinalis*, the regression of root length density (RLD) with penetration resistance and soil porosity as described by the equation given in Fig. 1 was significant at 0.05 level (R^2=0.56). Similarly, in the case of *P. incarnata* (Fig. 2), multiple linear regression of root mass density (RMD) with root surface density (RSD) and penetration resistance was also significant (R^2=0.894, p<0.01).

Concerning the AMF root colonization (Tab. 1 and Tab. 2) of *H. officnalis* and *P. incarnata* plants, there were significant differences between the irrigation treatments. The highest AMF root colonization was found under the IR2 treatment (27.75% and 47% for *H. officnalis* and *P. incarnata* plants). The AMF root colonization was lower in *H. officnalis* crop than that in *P. incarnata* crop. According to Chandra *et al.* (2010) mycorrhiza root colonization enhance the yield of many medicinal plants. In *H. officnalis* crop, the AMF root colonization had positive and significant correlation (Tab. 3) with root length density (r=0.81, p<0.01). Also, the AMF root colonization had negative and significant correlation with penetration resistance of soil. Moreover, in *P. incarnata* crop AMF root colonization had positive and significant correlation with soil porosity and root surface density (r=0.90, p<0.01 and r=0.75, p<0.05, respectively).

Tab. 3. Correlations coefficients between soil properties and root parameters of *Hyssopus officinalis* experiment. Marked correlations are significant at p < 0.05.

	Soil Porosity (%)	Penetration Resistance (Mpa)	MWD (mm)	RMD (g/cm^3)	RLD (cm/cm^3)	RSD (mm^2/cm^3)	AMF (%)
Soil Porosity (%)	-	-	-	-	-	-	-
Penetration Resistance (Mpa)	**-0.82**	-	-	-	-	-	-
MWD (mm)	**0.86**	**-0.93**	-	-	-	-	-
RMD (g/cm^3)	**0.85**	**-0.81**	**0.73**	-	-	-	-
RLD (cm/cm^3)	**0.82**	-0.63	0.58	**0.74**	-	-	-
RSD (mm^2/cm^3)	**0.75**	-0.59	**0.71**	**0.77**	0.60	-	-
AMF (%)	0.67	**-0.85**	**0.81**	0.56	**0.72**	0.45	-

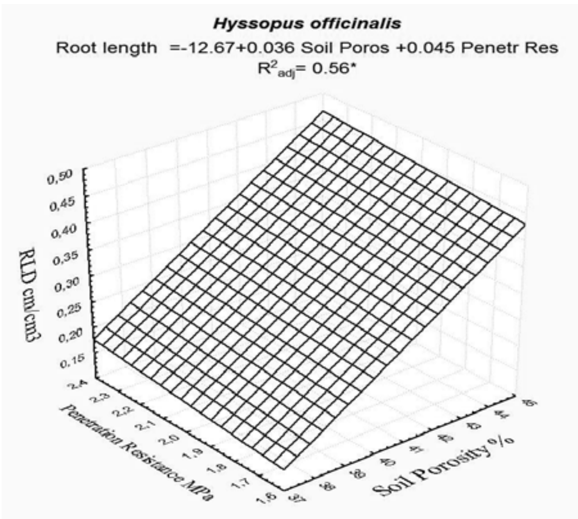

Fig. 1. Multiple linear regression of root length density (RLD), penetration resistance and soil porosity of *Hyssopus officinalis*.

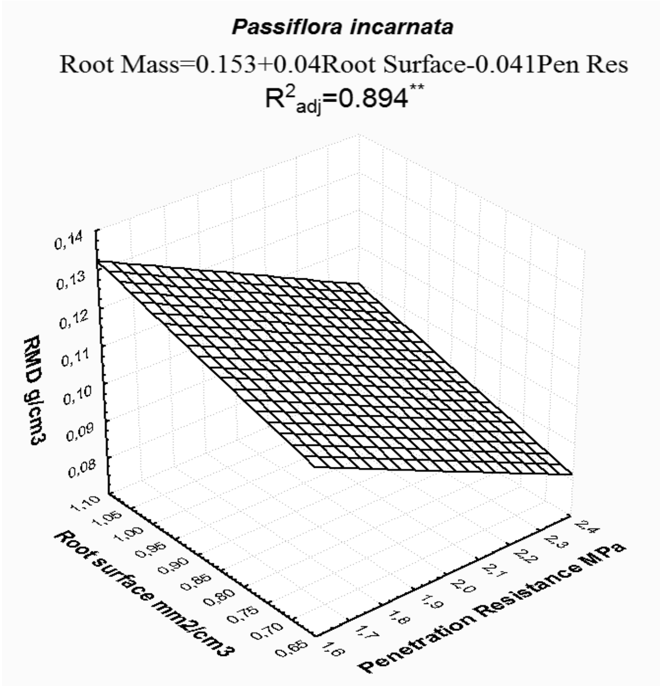

Fig. 2. Multiple linear regression of root mass density (RMD), root surface density and penetration resistance of *Passiflora incarnata*.

Tab. 4. Correlations coefficients between soil properties and root parameters of *Passiflora incarnata* experiment. Marked correlations are significant at $p < 0.05$.

	Soil Porosity (%)	Penetration Resistance (Mpa)	MWD (mm)	RMD (g/cm^3)	RLD (cm/cm^3)	RSD (mm^2/cm^3)	AMF (%)
Soil Porosity (%)	-	-	-	-	-	-	-
Penetration Resistance (Mpa)	-0.44	-	-	-	-	-	-
MWD (mm)	0.49	**-0.72**	-	-	-	-	-
RMD (g/cm^3)	0.57	**-0.93**	**0.83**	-	-	-	-
RLD (cm/cm^3)	0.36	-0.68	**0.85**	**0.71**	-	-	-
RSD (mm^2/cm^3)	0.66	**-0.76**	0.68	**0.86**	0.58	-	-
AMF (%)	**0.90**	-0.62	0.70	**0.76**	0.55	**0.75**	-

CONCLUSION

In the present study it was shown that an increase of irrigation rate resulted to a significantly higher growth and productivity of the medicinal plants *H. officinalis* and *P. inarnata*, by means of a positive effect on all the determinant parameters of root growth. Most of these parameters were strongly correlated, while mycorrhiza root colonization was also positively affected with an already proven beneficial impact on the yield of many medicinal plants.

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